Accepted: 13 June 2017

DOI: 10.1002/jcla.22298

RESEARCH ARTICLE

WILEY

Multiplex PCR scheme for variant plasmid mediated class C β-lactamase typing

Le Van Chuong[1,2] | Virapong Prachayasittikul[1] | Chartchalerm Isarankura Na Ayudhya[1] | Ratana Lawung[1]

[1]Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand

[2]Department of Microbiology, Faculty of Medicine, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam

Correspondence
Ratana Lawung, Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.
Email: ratana.law@mahidol.ac.th

Funding information
The Office of the Higher Education Commission, Mahidol University under the National Research Universities Initiative

Background: An increasing of prevalence and diversification of plasmid-mediated AmpC (pAmpC) has been emerged worldwide. The incidence of pAmpC resulted in increasing β-lactamase production and conferred resistance to almost all β-lactam antibiotics excluding carbapenems. The lack of standard method for pAmpC identification and classification exert a challenge in epidemiological surveillance and infection control practices.

Methods: A robust, single tube multiplex PCR has been developed to classify six different pAmpC groups including CIT (CMY-2 like, LAT and CFE), ECB (ACT, MIR), MOX & CMY-1 like, DHA, ACC, and FOX. The developed method was optimized and validated by testing of sensitivity and specificity.

Results: Developed method can detect crude extracted DNA template at nano-scale (2.5 ηg) and has high discriminatory power as compared to phenotypic and commercial genotypic method.

Conclusion: The developed method can be utilized for tracking the changes of clinically important resistance patterns and further investigation of occurrence and distribution of plasmid-mediated AmpC types.

KEYWORDS

cefoxitin resistance, Enterobacteriaceae, genotypic method, multiplex PCR, Plasmid-mediated AmpC β-lactamases

1 | INTRODUCTION

Enterobacteriaceae are the most common pathogens causing community- and hospital-acquired infections [1] and the antibiotic resistance Enterobacteriaceae have been reported worldwide. The infection tends to exert hospital burden for both health care institution and patient.[2] The major driving force of antibiotic resistance within this family is the production of broad spectrum β-lactamases including extended spectrum β-lactamases (ESBLs), plasmid mediated AmpC cephalosporinases (pAmpC), and carbapenemases. Furthermore, extended spectrum cephalosporin resistance (ESC-R) Enterobacteriaceae phenotype may harbor one or many types of broad spectrum β-lactamases and may also harbor multiple β-lactamase genes of the same classes of β-lactamases in one organism.[3] Because of these β-lactamase genes are mediated on plasmid, the resistance genes were easily transferred to other bacterial cells, species, and genus. These results inferred to the increasing rates of ESC-R Enterobacteriaceae.

Plasmid-mediated AmpC β-lactamase genes are descended from the chromosomal *ampC* genes of several organisms such as *Citrobacter fruendii*, *Enterobacter cloacae*, *Hafnia alvei*, and *Morganella morganii*.[4] Because of lack association with regulation genes (*ampR*), pAmpC is usually overexpression.[5] Although less common than ESBLs, plasmid AmpC β-lactamases (pAmpC) have been reported worldwide with various prevalence rates. These enzymes confer resistance to penicillins, first to third generation cephalosporins, cephamycins, monobactams, and also poorly inhibited by the commercially available β-lactamase inhibitors such as clavulanic acid. Up to date, at least eight classes of pAmpC have been characterized.[4,6] The name of pAmpC β-lactamases

were nominated randomly without typical of β-lactamases nomenclature, relying on resistance produced to cefoxitin (FOX), cephamycins (CMY), latamoxef (LAT), and moxalactam (MOX), to the type of β-lactamases for example AmpC type (ACT) or Ambler class C (ACC), and on the original location of detection, such as the Miriam hospital in Providence, Rhode Island, USA (MIR-1) or Dhahran hospital in Saudi Arabia (DHA).[7] The occurrence of pAmpC resulted in increasing of β-lactamase production and conferred broad resistance to almost β-lactam antibiotics excluding carbapenems.[8] Production of pAmpC enzymes also present challenges to susceptibility testing since it can mask the phenotypic detection of ESBLs and class A carbapenemases.[8] Furthermore, multiple β-lactamases (eg, ESBL-pAmpC enzymes and ESBL-AmpC-carbapenemase enzymes) within one organism has been characterized.[9] False negative result of producers can impede surveillance and infection control practices. Although several phenotypic methods have been proposed for AmpC detection, none of them has been approved by the Clinical and Laboratory Standards Institute (CLSI) or other criteria. Consequently, molecular method is a good choice of method to identify and differentiate between plasmid mediated AmpC (pAmpC) and chromosomal mediated AmpC (cAmpC).[1,2,6] To cope with the increasing diversity of pAmpC, molecular detection of pAmpC typing must be developed for further epidemiological survey. The aim of this study was to develop a multiplex PCR (mPCR) for rapid identification and molecular typing of pAmpC for further investigation of the prevalence and molecular epidemiology of pAmpC in cefoxitin resistant (FOX-R) Enterobacteriaceae isolates.

2 | MATERIALS AND METHODS

2.1 | Bacterial isolates

A total of 30 non-duplicated Enterobacteriaceae isolates which phenotypically presented with different characteristics of susceptibility to cefoxitin (FOX), third generation cephalosporin (3rd Ceph), and carbapenem were utilized in specificity testing of developed method (Table 1). Furthermore, 28 Enterobacteriaceae isolates (*E. coli* [*n*=16]; *K. pneumoniae* [*n*=7]; *Enterobacter spp.* [*n*=4]; and *P. rettgeri* [*n*=1]) of FOX-resistance and 3rd Ceph-resistance collected from Nakhon Pathom Regional Hospital – Nakhon Pathom – Thailand in late 2014 were involved to validate the performance of developed method compared to ampC ID kit (Streck, Omaha, NE, USA).

2.2 | Phenotypic detection of ESBL and carbapenemase production

The production of ESBLs was identified by combination disk test using ceftazidime (CAZ), ceftazidime/clavulanic acid (CAZ-CA), cefotaxime (CTX), and cefotaxime/clavulanic acid (CTX-CA; 30/10 μg) disks. Phenotypic carbapenemase confirmatory test was done by Modified Hodge Test (MHT). The tests were performed by following the instruction of the CLSI and *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains for quality control.[10]

TABLE 1 Clinical isolates used for specificity testing and validation method

Antimicrobial phenotype[a]	Number of isolates
1. 3rd Ceph-S; FOX-S; carbapenem-S	10
2. 3rd Ceph-R; FOX-S; carbapenem-S	10
3. 3rd Ceph-S; FOX-R; carbapenem-S	10

FOX, cefoxitin; S, susceptibility; R, resistance.
[a]3rd Ceph: third generation cephalosporin.

2.3 | Cephalosporinase AmpC detection

2.3.1 | Phenotypic AmpC detection

Phenotypic detection of AmpC production was performed by disk potential test.[11] Briefly, the cefotaxime (CTX) and ceftazidime (CAZ) disks with or without 300 μg of 3-aminophenylboronic acid (APB) were utilized to detect AmpC β-lactamases producing Enterobacteriaceae. The APB is a boronic acid derivative, one of the specific inhibitors of class C β-lactamases. The increasing of ≥5 mm in the growth-inhibitory zone diameter of CAZ-APB and CTX-APB compared with the zone diameter of CTX or CAZ disk alone was considered a positive result for AmpC β-lactamases production.[11]

2.3.2 | Genotypic detection

A single tube multiplex PCR was developed to identify six families of *ampC* genes including MOX+CMY-1 like (1), ECB (ACT, MIR) (2), DHA (3), ACC (4), CIT (CMY-2 like, LAT and CFE) (5), and FOX (6). The sensitivity and specificity were evaluated by comparison of tested results with commercial ampC ID kit (Streck, Omaha, NE 68145, USA).

2.3.3 | DNA extraction

The simple boiling method was applied to extract the DNA from Enterobacteriaceae isolates. Three to five overnight bacterial colonies on MacConkey agar were suspended in sterile distilled water to a turbidity of 0.5 McFarland and the bacterial cell suspension was heated at 95°C in 10 minutes. After centrifugation, the supernatant of each sample was used as DNA template without further purification.

2.3.4 | Designed primers

All pAmpC sequences recorded at Lahey Clinic website (http://www.lahey.org/Studies/) were collected from NCBI database (http://www.ncbi.nlm.nih.gov/nuccore/). Then, ClustalW multiple alignment function of Bioedit software was applied for alignment to check the alteration as well as homology of specific genes. Primer3 online software (http://bioinfo.ut.ee/primer3-0.4.0/) was used to pick up the primers. The primers were designed to classify six different groups including CIT (CMY-2 like, LAT and CFE), ECB (ACT, MIR), MOX & CMY-1 like, DHA, ACC, and FOX. The CDeg1 (5′-CARACSCTGTTYGAGMTRGG-3′) and

TABLE 2 Primer sequences used in multiplex PCR

Primer name	Primer sequence (5′-3′)[a]	Target genes	Expected amplicon size (bp)	References
CDeg1	CARACSCTGTTYGAGMTRGG	CMY, LAT-1, CFE-1, ACT, MIR, MOX, FOX	800[b]	12
CDeg2	GCCAAAICCRYYRGTSGAGCC			
MOX-F	GATCCGYCTTTGACARCATC	MOX, CMY-1 like[c]	665	This work
ECB-R	TYGCCATATCCTKSAYGTTRG	ACT, MIR [d]	526	
CIT-F	CAGCCDSGTTCARGADAAAA	CMY-2 like, LAT, CFE[e]	250	
FOX-R	CAAACCACCCGCACTGTAG	FOX[f]	181	
DHA-F	CGTTACAGGTRCCGGAHG	DHA[g]	346	
DHA-R	CAGCGCAGCATATCTTTTGA			
ACC-F	AYTATGCKTGGGGCTAYAAC	ACC[h]	422	
ACC-R	CATCACGATMCCCATCTTYT			

[a]R=A, G; S=C, G; Y=C, T; K=G, T; D=A, G, T and I is inosine.
[b]MOX-CMY-1 like=789-792; FOX=798; CMY-2 like, ACT-MIR=801.
[c]MOX1-12, CMY1, CMY8-11, CMY19.
[d]ACT1-10, ACT12-38; MIR1-19.
[e]CMY2-7, CMY12-18, CMY20-140; LAT1; CFE1.
[f]FOX 1-10, 12.
[g]DHA1-7, DHA9,10, DHA12-23.
[h]ACC1-5.

CDeg2 (5′- GCCAAAICCRYYRGTSGAGCC-3′) primers[12] were utilized to detect several genes encoding pAmpC β-lactamases. The forward CDeg1 primer was utilized in combination with ECB and FOX reverse primers for amplification of ECB and FOX genes while the CIT and MOX forward primers were designed and used with the CDeg2 reverse primer for amplification of CIT and MOX genes. Furthermore, the primer sets for DHA and ACC genes were also designed (Table 2).

2.3.5 | Optimized PCR condition

Amplification was performed in one PCR reaction tube. The PCR reaction was optimized in a total 20 μL reaction mixture containing 2 μL PCR buffer (10×), 1,2 μL $MgCl_2$ (25 mmol/L), 2 μL dNTPs (2 mmol/L each), a variable concentration of primers (Bio Basic Inc., Canada) including 20 ρM of AmpC-F and AmpC-R, 4 ρmol/L of MOX-F, ECB-R, ACC-F, ACC-R, DHA-F, and DHA-F, 8 ρM of CIT-F, 2 ρM of FOX-R, and 0.5 U i-Taq™ DNA Polymerase (iNtRON Biotechnology, Kyungki-Do, South Korea), 2 μL of template and finally an amount of sterile distilled water. Amplification was performed on MJ Research PTC-200 Gradient Peltier Thermal Cycler (Bio-Rad Laboratories, San Diego. CA, USA) by following thermal cycling conditions: one cycle of initial denaturation at 94°C in 3 minutes, then 35 cycles consisting of 20 seconds at 94°C, 15 seconds at 60°C, and 45 seconds at 72°C, and final extension step 3 minutes at 72°C. The amplicons were determined by electrophoresis on a 2% agarose gel (Research Organics INC., USA) and stained with ethidium bromide (0.5 μg/mL). A Genesnaph (Syngene, Frederick, MD, USA) was employed for PCR band recognition and imaging.

2.3.6 | Validation of mPCR

The sensitivity of mPCR assay was reported via the limit of detection (LOD). Suspension of known carrying pAmpC strains were lysed and measured the DNA concentration using NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Each known DNA templates were diluted to get concentration of 10, 5, 2.5, 1.25, 0,625, and 0.3125 ηg/μL and used in the PCR reaction. The lowest concentration of template that still giving positive result by displaying all patterns band obtained was reported as LOD.

The specificity of our developed method was verified via 30 clinical isolates of various susceptibility patterns (Table 1) and the accuracy of plasmid AmpC identification was verified by a comparison method. Twenty-eight cefoxitin and 3rd-cephalosporin resistance Enterobacteriaceae collected from Nakhon Pathom Regional Hospital – Nakhon Pathom – Thailand in late 2014 were identified AmpC β-lactamase production via both phenotypic method and genotypic methods, our developed method and commercial ampC ID kit (Streck). The commercial ampC ID kit was performed according to the instructions of the manufacturer. The different results between developed method and commercial ampC ID kit were identified pAmpC type by sequencing. Briefly, the partial ECB genes were amplified by PCR using CDeg1 and CDeg2 primers (Table 2). The PCR products (800 bp) were purified and then sent for sequencing.

3 | RESULTS

Our mPCR for pAmpC typing was successfully developed and optimized. The sensitivity of the mPCR was determined with the known DNA templates at different concentrations and the limit of detection (LOD) within 35 cycles was defined at 1.25 ηg/μL of DNA template (2.5 ηg per reaction) (Figure 1). No cross-reaction was found when tested with 30 clinical Enterobacteriaceae isolates in Table 1. By adding of CDeg1 and CDeg2 primers (primer

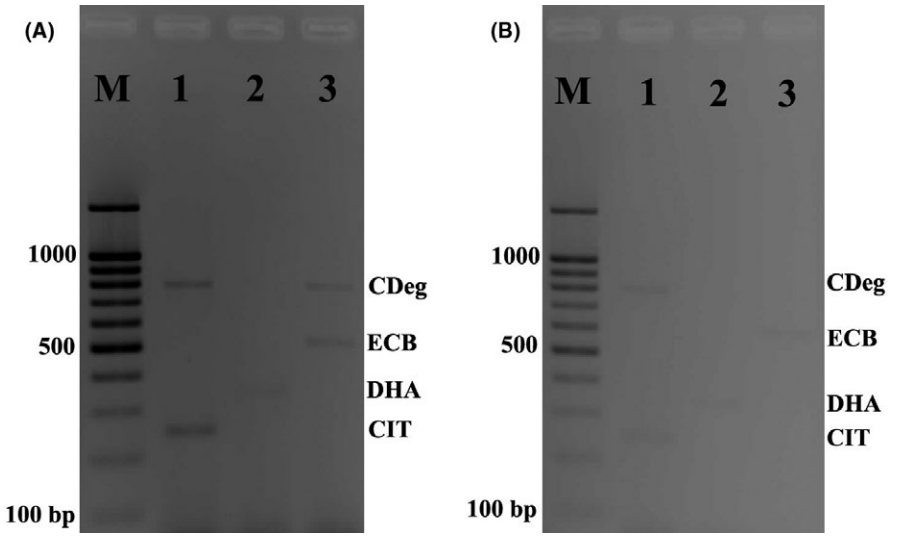

FIGURE 1 The sensitivity testing of developed method. The sensitivity of developed method was evaluated by testing with DNA templates at concentration 1.25 ηg/μL (A) and 0.625 ηg/μL (B). Lane 1 presented CIT (CMY-2 like, LAT and CFE), lane 2 presented DHA, lane 3 exhibited ECB (ACT/MIR), and lane M was 100 bp plus 1.5 kbp DNA ladder

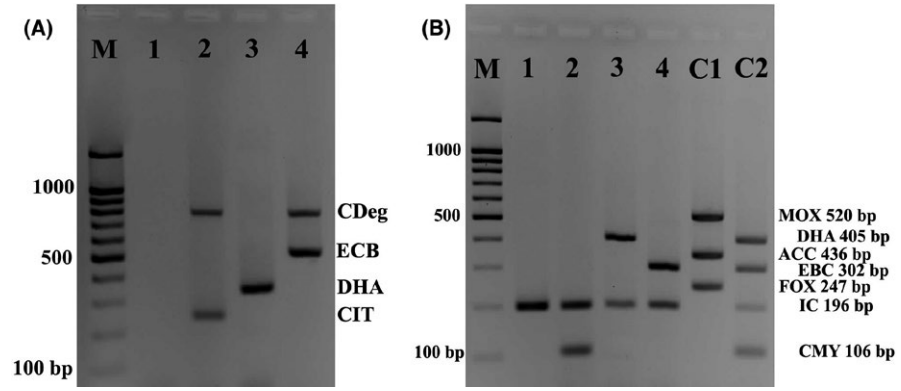

FIGURE 2 Correlation between developed method (A) and ampC ID kit (B). Lane 1 was a negative control (ATCC 25922 tested isolate), lane 2 presented CIT (CMY-2 like, LAT and CFE) gene, lane 3 presented DHA gene, and lane 4 exhibited ECB (ACT/MIR genes). C1 and C2 are controls, IC band is internal control (16S rRNA) and lane M was 100 bp plus 1.5 kbp DNA ladder

set for CMY, LAT-1, CFE-1, ACT, MIR, MOX, and FOX), the finding of each plasmid types in the reaction could be confirmed. Thus, plasmid AmpC carrying isolates showed at least two bands except for isolate carried only DHA alone (Figure 2A). The 28 cefoxitin and third cephalosporin resistant Enterobacteriaceae were phenotypic characterization of ESBL and carbapenemase production. Eighteen isolates were identified as ESBL producers and two isolates were defied as carbapenem resistant Enterobacteriaceae (CRE). This collection was also tested for AmpC production via phenotypic method, ampC ID kit, and our method. The AmpC β-lactamase was detected in 16 isolates and the pAmpC genotypes were found in 18 and 21 isolates by ampC ID kit and developed method, respectively. Fourteen of 16 AmpC positive phenotypic isolates carried plasmid AmpC β-lactamases. All positive plasmid AmpC isolates with commercial ampC ID kit was also detected with our developed method. Furthermore, three of 10 pAmpC negative isolates by commercial ampC ID kit were positive with ECB (ACT, MIR) genes via our system (Figure 2 and Table 3). The sequencing data of these three isolates were confirmed that all three ECB sequences belonged to ACT type, which were possibly identified only by our developed method.

4 | DISCUSSION

An increasing diversification of pAmpC genes has been reported in Enterobacteriaceae worldwide.[4,6] Differentiation of pAmpC β-lactamase producing isolates from other broad spectrum β-lactamase expressing Enterobacteriaceae is essential to address surveillance and epidemiology as well as hospital infection control issued associated with transferable resistance mechanisms. Unlike ESBLs, AmpC enzymes are not inhibited by β-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam), except for avibactam.[13] Since AmpC enzymes are encoded by both chromosome and plasmid, plasmid types are related to broad spectrum β-lactam resistance phenotype. It is the challenge to identify plasmid carrying AmpC isolates. Furthermore, the production of dual or multiple enzymes that showed different characteristics in one isolate may hinder detection by phenotypic method[13] Among seven AmpC producing isolates that showed negative by phenotypic method but positive by molecular methods, six isolates produced ESBLs and one isolates produced carbapenemase, respectively. In addition, phenylboronic acid method has good specificity (99%) but low sensitivity.[14] From these reasons referred that only 16 isolates were identified as AmpC producers by phenotypic

TABLE 3 Plasmid mediated AmpC β-lactamase among 28 cefoxitin and third generation cephalosporin resistant Enterobacteriaceae isolates characterized by commercial ampC ID kit and developed mPCR method

pAmpC types (ampC ID kit)	pAmpC types (Developed method)							
	CDeg[a]	CIT[b]	ECB[c]	DHA	ACC	MOX[d]	FOX	Negative[e]
CIT (*n*=11)	11	11						
ECB (*n*=1)	1		1					
DHA (*n*=6)	-			6				
ACC (*n*=0)	-				-			
MOX (*n*=0)	-					-		
FOX (*n*=0)	-						-	
Negative[e] (*n*=10)	3		3					7[f]

[a]CMY, LAT-1, CFE-1, ACT, MIR, MOX, and FOX.
[b]CIT (CMY-2 like, LAT, CFE).
[c]ECB (ACT, MIR).
[d]MOX (MOX, CMY-1 like).
[e]Isolate of negative result for pAmpC detection.
[f]Two of seven showed positive result via phenotypic method.

method, while the molecular methods were detected more. Among numerous phenotypic methods including biological based assays, inhibitor-based tests, and rapid chromogenic assay, the Tris-EDTA test and MAST ID D68C disk test were defined as high sensitivity and specificity.[14] Nevertheless, none of them are able to differentiate cAmpC and pAmpC as well as more than one pAmpC enzymes in an isolate.[9] Several molecular methods have been developed and the method of Pérez-Pérez et al.[15,16] was commercially applied as ampC ID kit. Then, they also developed a multiplex Real-Time PCR;[15,17] however, this method is expensive and not suitable for the laboratories where real-time thermal cycler are not available.

The strategy of our method using same forward primers or reverse primers offers low price and convenient for our mPCR since all primers can be combined in one tube. Our developed method is sensitive and able to detect target genes at nano-gram level. Nevertheless, the DNA templates used in this study were a product from crude extraction, therefore the LOD of each gene should be much lower than the validated ones. Furthermore, our system showed both high sensitivity and specificity. All positive samples with ampC ID kit can be detected by our tested system and three more isolates can identify ACT/MIR β-lactamase. This results may result of our primer design cope with more variants of ACT/MIR -AmpC types while the ampC ID kit can amplify only ACT-1, -2, -8, -12 and MIR 1-3, 6-7 and -8.[16] Based on this result, it is indicated that most of Enterobacteriaceae isolated from Thailand possess new variant ACT/MIR genes. In addition to DHA-type β-lactamase that was highly prevalent in the Asia-Pacific region[9] cannot be detected by CDeg1 and CDeg2 primers,[12] CDeg and DHA primer sets are needed in plasmid mediated AmpC screening and our mPCR system is benefit for identification of AmpC plasmid types in clinical isolates.

In conclusion, we presented a new tool for pAmpC typing that has high discriminatory power for pAmpC classification and coped with all presented pAmpC types. In the absence of CLSI guidelines of AmpC detection, our mPCR offers a reliable and alternative tool for pAmpC epidemiological surveillance. This new method is very suitable for further investigation of prevalence and distribution of AmpC plasmid types in Thailand and other countries and can be used for tracking the changes of clinical important resistant patterns.

ACKNOWLEDGMENTS

This study and Chuong Le Van were partially supported by the Office of the Higher Education Commission, Mahidol University under the National Research Universities Initiative. A special thanks to all staffs in the Department of Microbiology, Nakhon Pathom Regional hospital for supplying Enterobacteriaceae isolates.

REFERENCES

1. Reuland EA, Halaby T, Hays JP, et al. Plasmid-mediated AmpC: prevalence in community-acquired isolates in Amsterdam, the Netherlands, and risk factors for carriage. *PLoS ONE*. 2015;10:e0113033.
2. Reuland EA, Hays JP, de Jongh DMC, et al. Detection and occurrence of plasmid-mediated AmpC in highly resistant gram-negative rods. *PLoS ONE*. 2014;9:e91396.
3. Nordmann P. Carbapenemase-producing Enterobacteriaceae: overview of a major public health challenge. *Med Mal Infect*. 2014;44:51-56.
4. Shahid M, Sobia F, Singh A, et al. AmpC β-lactamases and bacterial resistance: an updated mini review. *Rev Med Microbiol*. 2009;20:41-55.
5. Harris PN, Ferguson JK. Antibiotic therapy for inducible AmpC beta-lactamase-producing Gram-negative bacilli: what are the alternatives to carbapenems, quinolones and aminoglycosides? *Int J Antimicrob Agents*. 2012;40:297-305.
6. Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev*. 2009;22:161-182.
7. Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type beta-lactamases. *Antimicrob Agents Chemother*. 2002;46:1-11.
8. Park YS, Yoo S, Seo M-R, Kim JY, Cho YK, Pai H. Risk factors and clinical features of infections caused by plasmid-mediated AmpC β-lactamase-producing Enterobacteriaceae. *Int J Antimicrob Agents*. 2009;34:38-43.
9. Sheng WH, Badal RE, Hsueh PR. Distribution of extended-spectrum beta-lactamases, AmpC beta-lactamases, and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results of the study for Monitoring Antimicrobial Resistance Trends (SMART). *Antimicrob Agents Chemother*. 2013;57:2981-2988.

10. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*, 26th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
11. Yagi T, Wachino J, Kurokawa H, et al. Practical methods using boronic acid compounds for identification of class C beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli*. *J Clin Microbiol*. 2005;43:2551-2558.
12. Kaye KS, Gold HS, Schwaber MJ, et al. Variety of beta-lactamases produced by amoxicillin-clavulanate-resistant *Escherichia coli* isolated in the northeastern United States. *Antimicrob Agents Chemother*. 2004;48:1520-1525.
13. Lupo A, Papp-Wallace KM, Sendi P, Bonomo RA, Endimiani A. Non-phenotypic tests to detect and characterize antibiotic resistance mechanisms in Enterobacteriaceae. *Diagn Microbiol Infect Dis*. 2013;77:179-194.
14. Ingram PR, Inglis TJ, Vanzetti TR, Henderson BA, Harnett GB, Murray RJ. Comparison of methods for AmpC beta-lactamase detection in Enterobacteriaceae. *J Med Microbiol*. 2011;60(Pt 6):715-721.
15. Geyer CN, Reisbig MD, Hanson ND. Development of a TaqMan multiplex PCR assay for detection of plasmid-mediated ampC beta-lactamase genes. *J Clin Microbiol*. 2012;50:3722-3725.
16. Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol*. 2002;40:2153-2162.
17. Geyer CN, Hanson ND. Multiplex high-resolution melting analysis as a diagnostic tool for detection of plasmid-mediated AmpC beta-lactamase genes. *J Clin Microbiol*. 2014;52:1262-1265.

How to cite this article: Chuong LV, Prachayasittikul V, Isarankura Na Ayudhya C, Lawung R. Multiplex PCR scheme for variant plasmid mediated class C β-lactamase typing. *J Clin Lab Anal*. 2018;32:e22298. https://doi.org/10.1002/jcla.22298